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AWARD NUMBER DAMD17-97-1-7260

TITLE: The Role of SHP1 as a Tumor Suppressor Gene in Human Mammary

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REPORT DATE: August 1998

TYPE OF REPORT: Annual

PREPARED FOR: Commander
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5400

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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	August 1998	Annual (1 Aug 97 - 31 Jul 98)

4. TITLE AND SUBTITLE

The Role of SHP1 as a Tumor Suppressor Gene in Human Mammary

5. FUNDING NUMBERS

DAMD17-97-1-7260

6. AUTHOR(S)

Chuan Gao

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University of Texas M.D. Anderson Cancer Center
Houston, Texas 77030

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5400

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

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13. ABSTRACT *(Maximum 200 words)*

To investigate the possible involvement of SHP1 in human mammary tumorigenesis, SHP1 was stably transfected into a human breast cancer cell line MDA-MB231, which has very low endogenous SHP1 level compared to normal breast epithelium. While the over-expression of SHP1 did not lead to any significant change in the proliferation rate of these cells, it appeared to enhance their ability in anchorage-independent growth as well as their potential of tumorigenesis in nude mice. The over-expression of SHP1 changed neither the tyrosine phosphorylation status of overall cellular proteins or EGFR upon EGF stimulation in MDA-MB231 cells, nor the activation patterns of MAP kinases and AKT upon EGF and PDGF stimulation, respectively, in NIH3T3 cells.

While more studies are necessary to further characterize the MDA-MB231 transfectants, such as their invasiveness, it has also been planned to study the involvement of each important domain of SHP1 in tumorigenesis with a series of SHP1 mutants generated in this laboratory.

14. SUBJECT TERMS

Breast Cancer SHP1 Protein tyrosine phosphatase

15. NUMBER OF PAGES

12

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Limited

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INTRODUCTION

The regulation of cell proliferation and differentiation is mediated by a complex network of signal transduction pathways. Protein phosphorylation is an important mechanism which cells use to pass signals from the extracellular environment to the nucleus. Proteins can be phosphorylated on tyrosine residues, a process catalyzed by protein tyrosine kinases (PTKs), and in a reverse action, dephosphorylated by protein tyrosine phosphatases (PTPs). Aberrant protein tyrosine phosphorylation plays an essential role in tumorigenesis. The activation of PTKs commonly promotes cell proliferation, and most of the oncogenes identified to date are either PTKs, their regulators, or their target proteins. In contrast to PTKs, relatively less research has been focused on the role that PTPs may play in tumorigenesis, although the functions of PTPs, which catalytically reverse the phosphorylation process mediated by PTKs, could be just as important as those of PTKs in cell growth regulation.

Structurally, PTPs can be divided into two groups: the transmembrane receptor PTPs and the intracellular non-receptor PTPs (1). SHP1 (also called SHPTP1, HCP, PTP1C and PTPN6) is a member of the non-receptor, intracellular PTP subfamily. SHP1 was first identified as a *src*-homology 2 (SH2) domain-containing PTP (2, 3), besides its consensus tyrosine phosphatase domain. It is expressed at high levels in hematopoietic cell lineages and at relatively lower levels in normal epithelial cells (2, 4). Studies by different laboratories have demonstrated associations between the SH2 domains of SHP1 and phosphorylated tyrosine residues of a variety of receptor PTKs (5-7). The main function of SHP1 in hematopoietic cells is commonly thought to be downregulation of the proliferative signals via dephosphorylating, and, hence inactivating, receptor tyrosine kinases which transduce these signals.

The functions of SHP1 in non-hematopoietic cells, on the other hand, are less studied and therefore remain largely unclear. There have been published studies reporting both positive and negative effects of SHP1 on proliferative signaling in a number of non-hematopoietic cell lines(8-10).The first suggestion that SHP1 played a role in tumorigenesis came from the study of loss-of-function mutations in the SHP1 gene which naturally occur in motheaten (*me*) mice. Consistent with the commonly-accepted role of SHP1 as a negative regulator of proliferative signaling in hematopoietic cells, mice with these mutations show hyperproliferation of certain types of hematopoietic cells, and consequently suffer from severe combined immunodeficiency, as well as systemic autoimmunity. Infiltration of the hyperproliferating neutrophils and granulocytes in the skin leads to patchy alopecia, --the "motheaten" phenotype. The *me* phenotype results

from total loss of functional SHP1 protein due to a premature stop codon generated by a frame-shift deletion within the N-terminus SH2 domain (11, 12). Some surprising findings have led to the initial speculation that SHP1 may act as a tumor suppressor: although homozygous *me/me* mice develop aberrant hematopoiesis and profound autoimmune disease, which cause hemorrhagic pneumonitis and consequent death by the age of 4-6 weeks (13, 14), one *me/me* mouse developed a metastatic mammary adenocarcinoma prior to death at 3 weeks of age. In contrast, the heterozygous *me/+* mice, which have a much longer life span, show a 60% chance to develop breast tumors by the age of 18 months, with SHP1 still expressed in their normal breast tissues but no longer in the tumors. All these observations suggest the possibility that SHP1 plays a role in mammary tumorigenesis.

The putative involvement of SHP1in human breast cancer has also been suggested by the following findings: **1**) SHP1 can associate with, and dephosphorylate, some epidermal growth factor receptor (EGFR) PTK family members (2, 12, 14, 15). The EGFR family, including EGFR and the products of the *erbB2/neu*, *erbB3* and *erbB4* genes, is a family of closely related growth factor receptor protein tyrosine kinases. These receptor PTKs strongly influence cellular growth through the ligand-stimulated tyrosine phosphorylation of intracellular substrates. The amplification of these genes has been observed in a variety of human carcinomas (16), and the amplification or overexpression of *erbB2/neu* is particularly associated with a poor prognosis in at least a third of human breast and ovarian cancers (17, 18); **2**) SHP1 can associate with *src* family PTKs. The *src* family includes nine members: *src*, *yes*, *fyn*, *lyn*, *lck*, *fgr*, *hck*, *blk* and *yrk*, that are intracellular PTKs involved in signal transduction pathways. Some of these PTKs are also implicated in oncogenic transformation in human mammary tumorigenesis (19, 20); In several studies, it has been shown that the activation of the EGFR family PTKs can cause an increase in Src PTK activity , whereas the inhibition of Src kinase activity can suppress the proliferative response mediated by the EGFR family PTKs (20-22). SHP1 is known to be a substrate for v-Src PTK; the overexpression of SHP1 can lead to decreased v-Src PTK activity and inhibited proliferation in fibroblasts (8). Although the exact nature and mechanism of the regulation of the EGFR family and of the *src* family PTK activity are yet to be understood, the involvement and significance of SHP1in such processes, and therefore in tumorigenesis, have been strongly suggested.

Several lines of evidence support the contention that SHP1 plays a role in the process of tyrosine phosphorylation-mediated signaling in epithelial cells and during human mammary tumorigenesis. This study is focused on the potential role of SHP1 in human mammary tumorigenesis and the mechanism by which SHP1 may function as a regulator of signaling in breast epithelium.

RESULTS

1. The expression and activity of SHP1 in human breast cancer cells.

The expression levels of SHP1 in a series of human breast cancer cell lines were determined by Western blotting analysis. In comparison to normal human breast epithelium and two nontumorigenic human breast epithelial cell lines, MCF 10A and 10F, most of the human breast cancer cell lines showed normal or slightly elevated levels of SHP1 expression, with the exceptions of the cell lines MDA-MB231 and MDA-MB435, which showed very low or undetectable levels of SHP1 expression, respectively.

The activity levels of SHP1 in these human breast cancer cell lines were determined in assays using PNPP as substrate following immunoprecipitation with anti-SHP1 antibody. The assay results showed that the levels of SHP1 activity in these cell lines corresponded with their levels of expression. No altered specific activity was found.

2. Stable over-expression of SHP1 in the MDA-MB231 human breast cancer cells.

SHP1, or an enzymatically inactive form of SHP1 was transfected into both the MDA-MB231 and MDA-MB435 cells using LipofectAmine reagent. Upon selection of stably transfected clones with G418, only the MDA-MB231 cells yielded numerous clones with SHP1 expression, whereas transfection of the MDA-MB435 cells failed to yield any clones with increased SHP1 expression in several attempts.

3. The effects of SHP1 when stably over-expressed in the MDA-MB231 cells.

A. The effects of SHP1 over-expression on proliferation rate.

The proliferation rates of the MDA-MB231 clones stably transfected with either vector alone, SHP1, or the inactive form of SHP1 were determined by the means of MTT assays. The results showed no significant difference in growth rates among all clones.

B. The effects of SHP1 over-expression on anchorage-independent growth.

The ability of the MDA-MB231 clones to grow in an anchorage-independent manner was determined by soft agar assays. The results showed that the over-expression of enzymatically active SHP1 in the MDA-

MB231 cells led to increased anchorage-independent growth, whereas the vector or the enzymatically inactive form of SHP1 failed to do so.

C. The effects of SHP1 over-expression on tumorigenecity in nude mice.

The tumorigenecity of the MDA-MB231 clones was determined in nude mice by injections of the cells in the mammary fat pad. The sizes of the tumors were measured over a period of two months. The results showed that only enzymatically active SHP1 rendered the cells increased tumorigenecity, while neither the vector nor the inactive form of SHP1 had any clear effects.

D. The effects of SHP1 over-expression on protein tyrosine phosphorylation in transfected MDA-MB231 cells upon EGF stimulation.

The changes in protein tyrosine phosphorylation status upon EGF stimulation in various MDA-MB231clones transfected with either vector, SHP1, or the inactive form of SHP1 were determined by Western blotting analysis using anti-phosphotyrosine antibody. The results showed no significant changes in the overall protein tyrosine phosphorylation level or EGFR tyrosine phosphorylation level upon EGF stimulation in all three types of clones, with various EGF concentrations and at different time points.

4. The effects of SHP1 when transiently over-expressed in NIH3T3 cells.

In an attempt to understand the role of SHP1 may play in signal transduction pathways, the possible effects of SHP1 on MAP kinase activation and AKT kinase activation were studied in transient transfection assays. These assays were performed in NIH3T3 cells due to the following reasons: 1. In the MDA-MB231 cells MAP kinases were constitutively activated whereas AKT failed to become activated upon stimulation, thus the clones could not be used in these studies. 2. NIH3T3 cells had undetectable level of endogenous SHP1 expression and could be more efficiently transfected.

A. The effects of SHP1 over-expression on MAP kinase activation.

NIH3T3 cells were transiently transfected with a plasmid containing HA-tagged ERK2, along with either the vector, SHP1, or the inactive form of SHP1, four times the amount of the HA-ERK2 plasmid. Upon EGF stimulation, HA-ERK2, presumably only expressed in cells also expressing the co-transfected second plasmid, was immunoprecipitated with anti-HA antibody and its activation determined by Western

blotting analysis using an antibody specifically recognizes the activated MAPKs. The results showed no significant effects on ERK2 activation from SHP1 over-expression when the cells were stimulated with EGF at various concentrations.

B. The effects of SHP1 over-expression on AKT activation.

NIH3T3 cells were transiently transfected with a plasmid containing HA-tagged AKT, along with either the vector, SHP1, or the inactive form of SHP1, four times the amount of the HA-AKT plasmid. Upon PDGF stimulation, HA-AKT, presumably only expressed in cells also expressing the co-transfected second plasmid, was immunoprecipitated with anti-HA antibody and its activation determined by Western blotting analysis using an antibody specifically recognizes the activated AKT. The results showed no significant effects on AKT activation from SHP1 over-expression when the cells were stimulated with PDGF at various concentrations.

CONCLUSIONS

1. In the MDA-MB231 human breast cancer cells, the over-expression of SHP1 did not alter cell proliferation rate.

2. In the MDA-MB231 cells, the over-expression of SHP1 resulted in increased anchorage-independent growth.

3. In the MDA-MB231 cells, the over-expression of SHP1 resulted in increased tumorigenecity in nude mice.

4. In the MDA-MB231 cells, the over-expression of SHP1 did not alter the tyrosine phosphorylation status of overall cellular proteins or EGFR upon EGF stimulation.

5. In NIH3T3 cells, the over-expression of SHP1 did not alter the pattern of ERK2 activation upon EGF stimulation.

6. In NIH3T3 cells, the over-expression of SHP1 did not alter the pattern of AKT activation upon PDGF stimulation.

BIBLIOGRAPHY

1. Fischer EH, Charbonneau H and Tonks NK: Protein tyrosine phosphatases: a diverse family of intracellular and transmembrane enzymes. *Science* 253:401-406, 1991.

2. Shen S, Bastien L, Posner B and Chretien P: A protein-tyrosine phosphatase with sequence similarity to the SH2 domain of the protein-PTKs. *Nature* 532:736-739, 1991.

3. Yi T, Cleveland JL and Ihle JN: Identification of novel protein tyrosine phosphatases of hematopoietic cells by PCR amplification. *Blood* 78:2222-2228, 1991.

4. Yi T, Cleveland JL and Ihle JN: Protein tyrosine phosphatase containing SH2 domains: Characterization, preferential expression in hematopoietic cells, and localization to human chromosome 12p12-13. *Mol. Cell. Biol.* 12:836-846, 1992.

5. Yi T and Ihle JN: Association of hematopoietic cell phosphatase with c-Kit after stimulation with c-Kit ligand. *Mol. Cell. Biol.* 13:3350-3358, 1993.

6. Yi T, Mui ALF, Krystal G and Ihle JN: Hematopoietic cell phosphatase associates with the interleukin 3 (IL-3) receptor B chain and downregulates IL-3-induced tyrosine phosphorylation and mitogenesis. *Mol. Cell. Biol.* 13:7577-7686, 1993.

7. Yi T, Zhang J, Miura O and Ihle JN: Hematopoietic cell phosphatase associates with erythropoietin (Epo) receptor after Epo-induced receptor tyrosine phosphorylation: identification of potential binding sites. *Blood* 85:87-95, 1995.

8. Matazaki T, Uchida T, Fujioka Y and Kasuga M: Src kinase tyrosine phosphorylates SHP1, a protein tyrosine phosphatase containing Src homology-2 domains that down-regulates cell proliferation. *Biochem. Res. Commun.* 204:874-881, 1994.

9. Su L, Zhao Z, Bouchard P, Banville D, Fischer EH, Krebs EG and Shen S-H: Positive effect of overexpressed protein-tyrosine phosphatase PTP1C on mitogen-activated signaling in 293 cells. *J. Biol. Chem.* 271:10385-10390, 1996.

10. You M and Zhao Z: Positive effects of SH2 domain-containing tyrosine phosphatase SHP-1 on epidermal growth factor- and interferon-r-stimulated activation of STAT transcription factors in Hela cells. *J. Biol. Chem.* 272: 23376-23381, 1997.

11. Tsui H, Siminovitch K, De Souza L and Tsui F: Motheaten and viable motheaten mice have mutations in the haematopoietic cell phosphatase gene. *Nature Genet. cs* 4:124-129, 1993.

12. Kozloeski M, Mlinaric-Rascan I, Feng G, Shen R, Pawson T and Siminovitch K: Expression and catalytic activity for the tyrosine phosphatase SHP1 is severely impaired in motheaten and viable motheaten mice. *J Exp Med* 178:2157-2163, 1993.

13. Shultz L and Green M: Motheaten, an immunodeficient mutant of the mouse. II. Depressed immune competence and elevated serum immunoglobulins. *J. Immunol.* 116:936-943, 1976.

14. Vogle W, Lammers R, Huang J and Ullrich A: Activation of a phosphotyrosine phosphatase by tyrosine phosphorylation. *Science* 259:1611-1614.

15. Tomic S, Greiser U, Lammers R, Kharitonenkov A, Imyanitov E, Ullrich A and Bohmer F-D: Association of SH2 domain protein tyrosine phosphatases with the epidermal growth factor receptor in human tumor cells. *J. Biol. Chem.* 270:21277-21284, 1995.

16. Prigent SA and Lemoine NR: The type 1 (EGFR-related) family of growth factor receptors and their ligands. *Prog. Growth Factor Res.* 4:1-24, 1992.

17. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A and McGuire WL: Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science* 235:177-182, 1987.

18. Slamon DJ, Godolphin W, Jones LA, Holt JA, Wong SG, Keith DE, Levin WJ, Stuart SG, Udove J, Ullrich A and Press MF: Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. *Science* 244:707-712, 1989.

19. Brickell PM: The p60c-src family of protein-tyrosine kinases: structure, regulation, and function. *Critical. Rev. in Onco.* 3:401-446, 1992.

20. Muthuswamy S and Muller W: Activation of the src family of tyrosine kinases activity in mammary tumorigenesis. *Adv. Cancer Res.* 64:111-123, 1994.

21. Muthuswamy S, Siegel P, Dankort D, Webster M and Muller W: Mammary tumors expressing the neu proto-oncogene possess elevated c-src tyrosine kinase activity. *Mol. Cell. Biol.* 64:111-123, 1994.

22. Siegel P, Dankort D, Hardy W and Muller W: Novel activating mutations in the neu proto-oncogene involved in induction of mammary tumors. *Mol. Cell. Biol.* 14:7068-7077, 1994.

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